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COMPLEMENT FIXATION WITH A SPECIFIC ANTIGEN IN ACUTE POLIOMYELITIS *

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Several attempts have been made by various investigators to obtain a specific antigen for complement fixation in poliomyelitis, so far without success.

We have obtained a specific antigen by digesting poliomyelitic virus with trypsin. A 5% suspension of brain and cord of monkeys that have died of poliomyelitis is filtered through a Berkefeld or Heim filter, sterile water being used as the menstruum. The trypsin is added in proportion of 1 : 50 and permitted to act at room temperature for three hours; 0.4% tricresol is then added to stop further action by the trypsin.[1]

The poliomyelitis antigens were diluted 1-5, the spinal fluid was used in 10 times the amount of the serum. The tubes were incubated as a rule for 2 hours in the water bath at 37 C. Earlier in the work, the antigen was used in less than one-fourth and later less than one-half of the anticomplementary dose. It is not inclined to be anticomplementary and was tested with each reagent alone, and these controls gave no hemolysis.

The bacterial antigens used as controls in our study of complement fixation in poliomyelitis were prepared by the methods employed by Miss M. A. Wilson in this laboratory, who made all the tests.

A saline emulsion of the dried organisms is made and the standard dose determined by titration with a homologous serum of known titer. The standard dose of each antigen contains 2 fixation units and one-fourth, or less, of the anticomplementary dose. The meningococcus tests are held for 30 minutes in the water bath at 37 C.

The tuberculosis and streptococcus tests are held for 2 hours in the water bath at 37 C. The antigen for the Wassermann tests was a cholesterinized alcoholic extract of guinea-pig-heart or ox-heart, made by Dr. Hadsopulos of the Wassermann department of Bellevue Hospital. The tubes were kept 1 hour in the water bath at 37 C.

In all of the tests 2 units of complement and 2 units of amboceptor were used, the cells having been sensitized with the amboceptor before they were added; 0.1 c.c. of a 5% suspension of sheep's corpuscles was used for indicator of the reaction of fixation.

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[1] For more elaborate description of the preparation, see Neustaedter and Banzhaf, Jour. Am. Med. Assn., 1917, 68, p. 1531.

No tests were made without controls of the serum or cerebrospinal fluid for anticomplementary action and for natural antisheep amboceptor.

All antigens were tested as to the anticomplementary dose, as to fixation with a homologous serum, and as to non-specific fixation with a heterologous serum.

The complement was a pool of the serum of from 6 to 10 pigs in every set of tests. The serum from each pig had been tested before pooling for natural antisheep amboceptor, for specific fixation with each control antigen-and-serum-combination to be used in the tests, for hemolysis of 0.1 c.c. of a 5% suspension of sheep's erythrocytes with the Wassermann unit of antisheep amboceptor.

All readings were made when the controls of the hemolytic system and of anticomplementary reaction in serums; fluids and antigens were completely laked. The tubes with positive reactions were then replaced in the water bath for one hour, to verify the accuracy of the reading. The reports were made on the first reading, but in most instances the reactions remained unchanged until the end of the hour.

In control of our work, we have used spinal fluid and serum of patients with other diseases than poliomyelitis. We also tested the same fluids and serums with various antigens at the same time, in order to be certain that we have a specific antigen.

We have examined 152 spinal fluids and 60 blood serums. The ages of the poliomyelitis patients ranged between 2½ and 23 years. The cases were frank and suspected, in the febrile and afebrile stages. The duration of the disease was from 1 to 40 days.

Of the 55 spinal fluids of poliomyelitis patients (Table 1) we obtained 2 ++++, 1 +++, 9 ++, 11 +, 12 ±, 7—, the balance were anticomplementary, due in some cases to their long standing after removal. The chemical and cytological results as well as the clinical symptoms in the doubtful, weakly and strongly positive cases coincided with the results as to fixation. Two of the negative cases proved on necropsy to be tuberculous meningitis; one negative case showed a ++++ Wassermann in the spinal fluid as well as in the blood.

Ed. S., a boy, aged 9, became ill with a temperature of 102 F. at 4 a. m. with malaise, and had a profuse diarrhea; the temperature rose to 104 F. within a few hours and toward evening he became stuporous and had convulsions. The spinal fluid was under high pressure at 9 p. m., contained 14 cells per c.mm., mostly lymphocytes, albumin + + +, globulin + + +, Fehling's reduction + +, no bacteria on smear or on culture; it was negative to cholesterin, tuberculous and meningococcus antigens. These results were obtained from examinations on the subsequent days. There was a slight left hemiparesis and a tendency to the Babinski phenomenon in the left foot. The pupillary reflex was normal, while the pupils were dilated. The deep and superficial reflexes were exaggerated. There was no Kernig or Macewen sign. No rigidity of the neck. The child could not be aroused from his stupor. He had a bloody stool. A tentative diagnosis of poliomyelitis was agreed on and 5 c.c. of immune

TABLE 1

SPINAL FLUIDS OF FRANK AND SUSPECTED CASES OF POLIOMYELITIS AND FIXATION WITH POLIOMYELITIS AND OTHER ANTIGENS

Number	Symptoms	Albumin	Cells	Globulin	Sugar	Poliomyelitis Antigen	Wassermann	Tubercle Antigen	Streptococcus Antigen	Meningococcus Antigen
1	Weakness in extremities; hydrocephalus	+++	Moderate increase	+++	+++	+++	0	0	0	0
2	Spastic hemiplegia	+++	Great increase	+++	+++	++	0	0	0	0
3	....	+	Normal	+	+++	++	0	0	0	0
4	Meningism; ataxia	+	Slight increase	+	+++	+	0	0	0	—
5	Deltoid paralysis; convulsions	+	Slight increase	+	+++	+	—	0	0	—
6	Meningism	+	Slight increase	+	+++	±	0	0	0	—
7	Paraplegia; recovered	+	Slight increase	++	+	—	0	0	0	0
8	Paresis	+	Moderate increase	+	+++	—	0	0	0	0
9	Deltoid paralysis	+	Slight increase	+	++	++	0	0	0	0
10	Measles; paresis	+	Slight increase	+	+++	+	0	0	0	0
11	Paraparesis	+	Slight increase	++	++	±	0	0	0	0
12	Meningism	+	Normal	+	+++	±	0	0	0	0
13	No data	++	0	++	++	+	0	0	0	0
14	No data	+	Slight increase	+	+++	±	—	—	±	±
15	Scarlet fever; transient blindness; meningism	+	Normal	+	+++	++	—	±	—	—
16	Meningism	+	Slight increase	+	+++	++	±	±	±	—
17	Flaccid paralysis	++	Slight increase	++	+++	++	—	±	—	—
18	No data	+	Slight increase	+	+++	++	0	0	0	0
19	No data	+	Slight increase	±	+++	±	+	±	±	++++
20	Paralysis of neck muscles	+++	Slight increase	+++	+++	++	—	±	—	++++
	Same case	++	Slight increase	+++	+++	±	—	±	—	—
21	No data	+	Slight increase	+	+++	+	0	—	0	0
22	Meningism	+	Normal	+	+++	+	—	0	0	0
23	Bulbar paralysis	++	0	++	+++	++++	0	0	0	0
24	Measles; pertussis; meningism	+	Normal	±	+	+	—	—	0	0
25	Left facial paralysis	+	Normal	±	+++	+	—	—	—	—
26	Paralysis of right arm	+	Normal	±	+++	+	—	0	0	0
27	Tuberculous meningitis	+++	Great increase	+++	+	++	±	±	±	—
28	Bulbar paralysis	+++	0	+++	+++	++++	—	—	0	0
29	Measles; foot drop	++	0	+	+++	—	0	0	0	0
30	Ataxia	+++	Slight increase	+++	+++	—	++++	0	0	0
31	No data	±	Slight increase	±	+++	±	—	—	0	0
32	Paraplegia	+++	Moderate increase	±	++	+	—	—	0	0
33	Paraplegia	......	Moderate increase	......	......	±	—	—	0	0
34	Paraplegia	+++	Great increase	+++	+	±	—	—	0	0
35	Paraplegia	++	Moderate increase	++	++	±	—	—	0	0
36	Deltoid paralysis	+	Slight increase	+	+++	±	—	—	0	0
37	Weakness of right arm; ataxia	±	Slight increase	±	+++	±	—	—	0	—
38	Tuberculous meningitis	+++	Great increase	++ ++	Trace	—	0	0	0	—
39	Convulsions; stupor	+++	Moderate increase	+++	++	—	—	—	0	—
40	Deltoid paralysis	++	Moderate increase	++	+++	+	0	0	0	0
41	Tuberculous meningitis	+++	Great increase	+++	+++	—	0	0	0	0

horse serum were injected intraspinally. Within 1½ hours a severe reaction followed, but after an injection of morphin (⅙ grain) the child became quiet. He seemed to be doing fairly well, when at 4:30 a. m. he died suddenly of respiratory failure.

The picture of this case does not coincide with poliomyelitis, and this is further confirmed by the negative reaction of the spinal fluid, since the 2 cases that gave a ++++ reaction died, and in every case with high fever we had ++. However, in view of the absence of a necropsy we have classed the case as one of poliomyelitis. The other negative cases were recovering, and it is possible that when cases recover, the fluids become negative.

Of 15 spinal fluids from cases of tuberculous meningitis (Table 2), 7 proved negative with the poliomyelitis antigen, 1 ±, and 1 ++, 6 were anticomplementary.

Of 20 spinal fluids of cases of epidemic cerebrospinal meningitis (Table 3), 13 were negative, 3 doubtful and 4 anticomplementary.

Of 22 spinal fluids of cases of tertiary syphilis, one gave a ++++ reaction with the poliomyelitis antigen, 1 +, 3 were anticomplementary, and the rest negative (Table 4).

Of 40 spinal fluids of normal and diverse pathologic conditions, one gave a ++++ reaction with the poliomyelitis antigen, 7 were anticomplementary, and the rest negative (Table 5).

Of 60 blood serums of various pathologic conditions, none gave a positive reaction with the poliomyelitis antigen (Table 6).

The results of the tests show that of 42 spinal fluids of frank and suspected cases of poliomyelitis, 23, or 53.5%, gave a positive reaction, 12, or 27.9%, gave a doubtful one, and 4, or 9.5%, a negative reaction. When we take into consideration the fact that the doubtful reactions were associated with clinical symptoms and cytological and chemical reactions in the fluids, showing unmistakably that an inflammatory process was going on in the meninges, we must place some value on these results.

We have had only 2 blood specimens from patients with poliomyelitis, and both were negative to the poliomyelitis antigen. The spinal fluid of 1 of these cases (1052) was + and the other (866) negative. Of course, no conclusion can be drawn from this. It is significant, however, that with 1 exception, Case 8972, which gave a ++++ reaction with the meningococcus antigen, all serums gave negative reactions with bacterial antigens. Further studies may bring more light into this question.

TABLE 2

SPINAL FLUIDS OF CASES OF TUBERCULOUS MENINGITIS AND FIXATION WITH POLIOMYELITIS AND OTHER ANTIGENS

Number	Cells	Albumin	Globulin	Sugar	Poliomyelitis Antigen	Wassermann	Tubercle Antigen	Streptococcus Antigen	Meningococcus Antigen
1	Great increase	+++	+++	+++	—	0	0	0	0
2	Blood	++++	++++	......	—	0	0	0	0
3	Great increase	+++	++++	Trace	—	0	0	0	—
4	Slight increase	++	++	+	—	0	0	0	0
5	Great increase	++++	++++	++++	±	—	±	±	—
6	Great increase	++	++	+	—	±	±	±	—
7	Moderate increase	++	++	+++	—	—	—	0	0
8	Great increase	+++	+++	+	++	±	±	±	—
9	Great increase	+++	+++	Trace	—	0	0	0	0

TABLE 3

SPINAL FLUIDS OF CASES OF EPIDEMIC MENINGITIS SHOWING REACTION TO POLIOMYELITIS ANTIGEN

Number	Cells	Albumin	Globulin	Sugar	Poliomyelitis Antigen
1	Blood	++++	++++	+++	—
2	Great increase	+++	+++	+	—
3	0	+	+	+++	±
4	Great increase	+++	+++	+	—
5	Moderate increase	++++	++++	+++	±
6	Great increase	++++	++++	++	—
7	Great increase	++++	++++	+++	—
8	Great increase	+++	+++	+	—
9	Great increase	+++	+++	+	—
10	Great increase	+++	+++	+	—
11	Great increase	+++	+++	Trace	—
12	Great increase	++++	++++	+	—
13	Great increase	+++	+++	—	—
14	Great increase	+++	++++	+	—
15	Great increase	+++	+++	+	—
16	Great increase	+	±	+++	±

TABLE 4

SPINAL FLUIDS OF CASES OF TERTIARY SYPHILIS SHOWING REACTIONS WITH CHOLESTERIN AND POLIOMYELITIS ANTIGEN

Number	Diagnosis	Wassermann	Poliomyelitis Antigen
1	Tabes	++++	—
2	Cerebrospinal lues	++++	±
3	Combined sclerosis	++++	±
4	Tabes	++++	±
5	Tabes	++++	±
6	Tabes	++++	++++
7	Tertiary lues	++++	+
8	Cerebrospinal lues	++++	—
9	Cerebrospinal lues	++++	—
10	Cerebrospinal lues	++++	—
11	Cerebrospinal lues	++++	±
12	Cerebrospinal lues	++++	—
13	Cerebrospinal lues	—	—
14	Tabes	++++	—
15	Tabes	—	—
16	Tertiary lues	—	—
17	Cerebrospinal lues	++++	—
18	Tabes	++++	—

TABLE 5

SPINAL FLUIDS OF MISCELLANEOUS CASES AND FIXATION WITH POLIOMYELITIS AND OTHER ANTIGENS

Number	Diagnosis	Cells	Albumin	Globulin	Sugar	Poliomyelitis Antigen	Wassermann	Tubercle Antigen	Streptococcus Antigen	Meningococcus Antigen
1	Normal	....	......	......	......	—				
2	Meningism	Normal	......	......	+++	—				
3	Childbirth	....	......	......	......	—	—			
4	Cerebral hemorrhage	....	......	......	......	—	—			
5	Meningitis	....	......	......	......	—	—			
8	Cerebral hemorrhage	....	......	......	......	—	—			
7	None	....	......	......	......	—	—			
8	Normal	—	—	—	+	—	—			
9	Tetany	....	......	......	......	—				
10	Normal	—	—	+	+	—	—			
11	Normal	—	—	—	+	—	—			
12	Normal	—	—	—	+	—	—			
13	Staphylococcus meningitis	Great increase	++++	++++	—	—				
14	Brain abscess	Great increase	++++	++++	Trace	—				
15	Brain hemorrhage	Great increase	+++	+++	+	—				
16	Tumor hypophysis	....	......	......	......	—	—			
17	Epilepsy	....	......	......	......	—	—			
18	Normal	....	......	......	......	—	—			
19	?	Great increase	+	+	+++	—	—	......	—	—
20	?	Normal	++	++	+++	—	......	......	—	—
21	Meningism	....	......	......	......	—	—			
22	Normal	Normal	±	±	+++	—	—	—	—	—
23	Meningism	Slight increase	+	+	+++	—	—			
24	Pertussis	Normal	+	±	+++	—	—	—		
25	?	Normal	+	±	+++	—	—	—	—	—
26	Staphylococcus meningitis	Great increase	+++	+++	++	—	—	—		
27	Meningism	Slight increase	+	+	+++	—	—	—		
28	Brain hemorrhage	Blood	++	++	+++	—	—	—		
29	?	Great increase	+++	+++	+	—	—	—	—	—
30	Meningism	Slight increase	++++	++++	+++	—	—	—	—	—
31	Meningism	Normal	±	±	+++	—	—	—	—	—
32	Interstitial nephritis	Normal	......	+	......	++++	—			
33	Pneumonia; meningism	....	......	......	......	—	—	—		

As controls, we have tested spinal fluids of normal and various pathologic conditions with the poliomyelitis antigen. Of 97 such fluids, 2 prove ++++, 1 ++, 1 +, 9 ±, and the balance negative. Cases 6 and 90, which were positive to the poliomyelitis antigen, gave a strongly positive Wassermann reaction. We explain this discrepancy by the fact that at that time we filtered our suspension through paper pulp and much of the lipoid substance remained in the filtrate. The reactions were in all probability due to the presence of the lipoid substance.

TABLE 6

SHOWING FIXATION OF BLOOD-SERUM WITH VARIOUS ANTIGENS

Diagnosis	Poliomyelitis Antigen	Wassermann	Tubercle Antigen	Streptococcus Antigen	Meningococcus Antigen
Chorea	—	—	—	0	—
Tabes	—	++++	—	0	—
Lues	—	++	0	0	—
Poliomyelitis	—	0	0	0	—
Epidemic meningitis	—	0	0	0	—
Poliomyelitis	—	0	0	0	—
General paresis	—	—	0	0	0
Nephritis	—	—	0	0	0
Mania	—	++++	—	—	—
Constitutional inferiority	—	±	—	—	—
Mania	—	—	—	—	—
Cerebrospinal lues	—	++++	—	—	—
Cerebrospinal lues	—	++++	—	—	—
Arteriosclerosis	—	±	—	—	—
Cerebrospinal lues	—	++++	—	—	—
Tertiary lues	—	++++	—	—	—
Secondary lues	—	++++	—	—	—
Gout	—	—	0	0	0
Pleuritis	—	—	0	0	0
Suppurative salpingitis	—	—	0	0	0
General paresis	—	—	0	0	0
Tertiary lues	—	++++	0	0	0
Cerebrospinal lues	—	++++	0	0	0
Cardiovalvular disease	—	—	0	0	0
Leukemia	—	—	0	0	0
Nephritis	—	—	0	0	0
Normal	—	—	0	0	0
Polyneuritis	—	—	0	0	0
Cardiovalvular disease	—	++++	0	0	0
Cardiac disease	—	—	0	0	0
Normal	—	—	0	0	0
Hemiplegia	—	—	0	0	0
Suppurative salpingitis	—	—	0	0	0
Cerebrospinal lues	—	+	—	—	—
Poliomyelitis	—	++++	±	—	—
Apoplexy	—	—	—	—	—
Nephritis	—	—	—	—	—
Normal	—	—	—	—	—
Cholelithiasis	—	—	—	—	—
Abortion	—	—	—	—	—
Pneumonia	—	—	—	—	—
Lues	—	±	—	—	—
Gonococcal arthritis	—	±	—	—	—
Cholelithiasis	—	—	—	—	—
Normal	—	—	—	—	—
Normal	—	—	—	—	—
Lues	—	++++	—	—	—
Nephritis	—	—	—	—	—
Brain tumor	—	—	—	0	0
Lues	—	+	—	0	0
Cardiovalvular disease	—	++++	—	—	—
No diagnosis	—	—	—	—	—
Tabes	—	++++	—	—	—
Hydrocephalus	—	—	—	—	—
Lues	—	++++	—	—	—
Salpingitis	—	—	—	—	—
Aortitis	—	++++	—	—	—
Epididymitis	—	—	—	—	++++
Alcoholism	—	—	0	0	0

We have since filtered all suspensions through Berkefeld or Heim filters and the results as recorded show the correct interpretation. All serums of syphilitic patients with a strongly positive Wassermann reaction were negative to the poliomyelitis antigen. Case 8991, which shows a ++++ reaction with our antigen, was diagnosed as an interstitial nephritis. The patient was 37 years old and had been only a few days in the hospital before he died. He had pain in the legs; the temperature ranged between subnormal and 101 F.; patellar reflexes sluggish, and achilles reflexes absent. At necropsy there was found interstitial nephritis, but the cerebral nervous system was not examined. The cells in the fluid were reported as 4 per c.mm. and the globulin as +; no Fehling's test or tests for albumin were made. In view of these incomplete findings no conclusions can be drawn.

CONCLUSION

It would appear that an antigen has been found, which probably is specific and fixes complement, hence of positive diagnostic value in poliomyelitis. This result is in accord with the production of immune bodies in a horse injected with poliomyelitic virus digested with trypsin as described.